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Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the San Bernardino Mountains, California

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Abstract

Decreased root biomass in forest trees in response to anthropogenic pollutants is believed to be one of the first steps in forest health degradation. Although decreased root biomass has been observed in controlled experiments, ozone effects on mature tree roots in natural stands has not previously been documented. Here we report standing root biomass of ponderosa pine at three sites in the San Bernardino Mountains distributed along a known, long-term pollution gradient of ozone and nitrogen deposition. Trees at each site were assessed for foliar ozone injury and below-ground attributes, in addition to other environmental factors known to influence root growth. During the period of peak root growth in the spring, root biomass at the least polluted site was 6–14 times greater than that observed at the most polluted site. Known differences in climatic and edaphic factors among the sites potentially contributing to the observed response were discounted as primary contributors to the response since in most cases the site differences would have driven the patterns of root growth in the opposite direction to that observed. Differences in biotic competitive interactions, also known to affect root growth, did not explain the observed pattern for the same reason. The data suggests that elevated ozone, high nitrogen deposition, and possibly other contributing factors such as soil acidification are primarily responsible for lowering root biomass in ponderosa pine stands in the San Bernardino Mountains. Published by Elsevier Science Ltd.

Keywords: Root biomass; Ponderosa pine; Ozone exposure; N deposition; Root carbohydrates

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1. Introduction

Over the last 40 years, the forests in the San Bernardino Mountains east of Los Angeles, California have been subjected to high levels of ozone and nitrogenous pollutants (Miller et al., 1989; Fenn and Bytnerowicz, 1993). A gradient of high to moderate ozone concentrations from the western to the eastern edge of the mountains is well documented (Miller et al., 1986; Table 1). Canopy health of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) to oxidant exposure has been measured in several ways over the last 20 years (Pronos et al., 1978; Miller et al., 1996), and injury has been clearly demonstrated at the western end of the mountain range, decreasing eastward with reduced pollution exposure (Miller et al., 1989; Miller and Rechel, 1998; Table 2). Loss of bole growth of ponderosa pine in response to oxidant exposure has also been demonstrated along this gradient (McBride et al., 1975; McBride and Miller, 1987). Elevated nitrogen deposition, soil acidification, and lower base saturation are associated with the highest oxidant exposure (Fenn and Bytnerowicz, 1993; Fenn et al., 1996).

Ponderosa pine is one of the most sensitive conifers in western North America to atmospheric ozone (Miller et al., 1983; Stolte et al., 1992). Carbon acquisition is lower in ponderosa pine exposed to elevated ozone both in chamber studies (Patterson and Rundel, 1989; Bytnerowicz et al., 1991), and in field studies in the San Bernardino Mountains (Coyne and Bingham, 1981; Coyne and Bingham, 1982; Grulke, 1998). In general, plants exposed to oxidant stress use resources for reparation activities at the expense of allocation to other plant parts (Barnes, 1972; Trebst, 1994). Retention of carbon resources in the foliage under moderate to high oxidant exposure has been interpreted as a preferential shift in biomass from the roots to the shoot. Chamber

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Table 1
Ozone concentrations from west to east (high to moderate pollution) across the San Bernardino Mountains east of Los Angeles, CA measured for 3 weeks at the end of July through early August in ppm-h on a 24 h basis

	Camp Osceola	Sky Forest	Camp Paivika	Source
1974	0.07	0.08	0.11	Miller et al., 1986
1975	0.07	0.07	0.11	Miller et al., 1986
1976	0.05	nd	0.10	Miller et al., 1986
1993	0.07	0.08*	0.09	This study
1994	0.07	0.09	0.09	This study

*Monitoring station was located at Strawberry Peak this year, 3 km W of Sky Forest.

Table 2
Foliar injury score across a pollution gradient from west to east in the San Bernardino Mountains

	Camp Osceola	Dogwood*	Camp Paivika	Source
1974	1.016	0.643	0.633	Miller et al., 1989
1978	1.262	0.643	0.506	Miller et al., 1989
1988	2.393	1.057	0.316	Miller et al., 1989
1994	1.425	nd	0.420	Miller et al., 1998

The score given here is the Forest Pest Management (FPM) index (Pronos et al., 1978; Miller et al., 1996) which uses a five point nominal rating system that identifies injury based on the most recent whorl of needles with the diagnostic chlorotic mottle symptoms of ozone.
A higher value indicates less ozone injury.
Trees measured at Barton Flats in the two studies (Miller et al., 1989; 1998) were in nearby plots, but were not the same trees.
*Dogwood Campground is 1 km w of Strawberry Peak site in this study.

experiments exposing ponderosa pine seedlings to ozone have demonstrated lower root biomass (Hogsett et al., 1989; Andersen et al., 1991, 1997; Miller et al., 1995). Decreased carbohydrate allocation to roots has also been observed in tree seedlings exposed to ozone (Tingey et al., 1976; Gorissen et al., 1991; Andersen and Rygiewicz, 1995). However, reduced resource allocation to, or retention by, roots in mature forest stands exposed to elevated ozone has only been hypothesized (Miller et al., 1982), not demonstrated. The ecological significance of reduced root biomass in forest trees is greater susceptibility to drought stress, increased insect attack, and greater wind throw probability. Under the influence of several stressors, tree mortality could result indirectly from high pollution loads.

The timing of resource allocation to growth throughout the tree is critical to interpreting the effects of environmental factors on root growth. Both above- and below-ground environmental conditions act to alter patterns of root growth and standing biomass (Kozlowski and Pallardy, 1997). Current photosynthate appears to be necessary for new root growth in conifers (van der Driessche, 1987), suggesting that conditions favorable for photosynthesis are needed to supply the roots with adequate carbon for growth. Ambient air temperatures influence the timing and magnitude of shoot carbon acquisition and growth, which affects the total carbohydrate pool size and the timing of its availability for allocation to root growth. Periods of most active root growth are often out of phase with shoot growth due to within-plant competition for carbon resources among sinks (van der Driessche, 1987). Below-ground, soil temperature affects root growth and respiration directly, but it also affects allocation processes throughout the plant via changes in sink strength (Gholz and Cropper, 1991). Low soil temperature (Kozlowski and Pallardy, 1997), nutrient availability (Magill et al., 1997), and moisture availability (Hermann and Petersen, 1969) have all been shown to affect root biomass and/or shift resource allocation between above- and below-ground tissues. In addition to oxidant exposure, biotic stresses such as canopy shading and tree density also alter root biomass via reduced photosynthate.

The current study was undertaken to describe patterns of root biomass across a pollution gradient in the San Bernardino Mountains, to link below-ground biomass patterns to above-ground attributes known to respond to oxidant exposure and N deposition, and to discuss the role of other abiotic and biotic factors important in influencing root biomass in ponderosa pine. The timing of above-ground growth was used to test for differences in phenology among sites. The relative differences in the oxidant exposure across the San Bernardino Mountains has been consistent for two decades (Table 1). In addition to pollution exposure, abiotic (soil temperature, nutrients, moisture, and acidification status) and biotic stressors (tree density and canopy shading) are quantified and discussed with respect to their role in influencing the observed differences in root biomass among sites. These factors were described, over a period of the preceding 5 years to root biomass measures, and are assumed to adequately describe site attributes across the pollution gradient.

2. Methods

2.1. Site and individual tree selection

Three sites along a pollution gradient were chosen for study within the mixed conifer zone, in the San Bernardino Mountains. Sites were very close to permanent plots of Miller et al. (1989): the Camp Paivika site in this study (34°14′05″N, 117°19′12″W) was 0.2 km S of Miller's Camp Paivika site, the Strawberry Peak site (34°14′00″N, 117°08′12″W) was 2 km E of Miller's Dogwood Campground site, and the Camp Osceola site (34°09′42″N, 116°51′00″W) was 0.5 km W of Miller's Camp Osceola site (Table 2). The ozone exposure was

Table 3
Morphological and physiological attributes of the six ponderosa pine sampled per site

	CO	SP	CP	*F*	*p*	Year
Tree age	45 ± 5	30 ± 4	39 ± 5	1.81	0.192	92
Nearest neighbor	1.6 ± 0.2	1.3 ± 0.1	2.2 ± 0.2	10.73	0.0001	92
Bole diameter	2.2 ± 0.2	3.9 ± 0.2	5.5 ± 0.3	16.77	0.0001	92
Branchlet diameter	5.9 ± 0.3	7.3 ± 0.3	7.2 ± 0.3	7.81	0.002	95
Whorls retained	4.4 ± 0.2	3.8 ± 0.3	2.8 ± 0.3	10.17	0.002	95
Foliar chlorotic mottle	2 ± 0	11 ± 2	28 ± 5	25.94	0.0001	95
% Foliar N	0.99 ± 0.03	1.20 ± 0.03	1.42 ± 0.05	31.57	0.0001	95
Chlorophyll	4.83 ± 0.26	4.98 ± 0.25	6.12 ± 0.42	4.92	0.023	95
Chlor a:b	1.05 ± 0.01	1.09 ± 0.03	1.02 ± 0.01	3.25	0.067	95

The mean and and 1 ± SE are given for six replicate trees per site; the *F* statistic and its significance (*p*) are given for the ANOVA performed across sites.

Tree age is given in years, distance to the nearest conspecific neighbor in m, bole diameter in cm, branchlet diameter in mm, number of whorls retained and percentage foliar chlorotic mottle in September, foliar N content in mid July as per cent of dry weight, and photosynthetic pigment content in mid July (total, in mg chlorophyll per g dry weight).

Foliar and branchlet attributes were taken on 1 year old tissue.

characterized across this gradient by Miller et al. (1982; 1986) based on 4 years of summer ozone concentration measurements at a subset of 18 sites transversing the west to east (high to moderate) pollution gradient.

Within each site, large plots were established (50×300 m), within which all ponderosa pine trees were aged, measured for bole diameter, distance to the nearest con- and inter-specific neighbor, percentage chlorotic mottle on the previous-year foliage, and number of whorls retained averaged for five branches. The six most average trees in each plot in the 21–60 year age class were chosen for a 5 year (1992–1997) intensive study based on these morphological characteristics (Table 3).

2.2. *Environmental monitoring*

At each site, soil temperature was sampled every 5 min and an hourly average was stored using a Campbell 21x[1] data logger in 1993 and 1994 at all sites. For the soil temperature, a 24 gage, copper-constantan thermocouple in triplicate was used with epoxy bead tips. Ozone concentrations were measured hourly with a Dasibi monitor (Model 1008) throughout the year, but presented here for 15 April to 15 October in both years. Sample air was drawn from approximately 3–4 m in height (varied depending on site), at approximately 2 m from a building where instrumentation was housed. Dust was filtered through a teflon filter prior to passing the sample air through the analysis tube. Ozone monitors were calibrated once per month during the summer months (May–October) with a National Institute of Standards and Technology-traceable, tertiary standard. The calibration of the monitors was checked annually with an external audit device certified by the Environmental Protection Agency.

Several pits were excavated at each site (approximately 50 cm×50 cm×1 m), and the most representative soil pit was chosen for description. The middle of each soil horizon was sampled for morphological and chemical analysis in mid July 1996. Total carbon and nitrogen was measured with a Carlo-Erba NA1500 C-N-S analyzer. Total exchangeable cations, and individual cations were determined after a 1 N ammonium acetate extraction (Na, Ca, Mg, K) on an ICP-OEC. Labile N (ammonium, nitrate) was measured after a 2 M KCl extraction. Ammonium was measured colorimetrically by an automated indophenol method (Keeney and Nelson, 1982). Nitrate was measured after reduction with copperized cadmium in combination with diazotization using a Technicon TRAACS 800 autoanalyzer. Total phosphorus was measured colorimeterically on the Technicon Autoanalyzer II after a $LiSO_4$ digest.

Pre-dawn needle water potential was used as an integration of soil drought stress experienced by the whole tree, monthly, in 1993, 1994 and 1995. Needle xylem water potential was measured on whole fascicles (Pallardy et al., 1991). The sheath was removed, a smooth surface was cut just distal to the petiole, and inserted within the pressure chamber (Pressure Measurement Systems, Corvallis, OR). Pressure was read to the nearest 0.05 MPa when apoplastic water first started bubbling at the cut fascicle surface, usually through sap.

2.3. *Above-ground growth*

Five primary branches were marked permanently for branch and needle growth in the lower third of the canopy of each tree chosen for intensive study. Primary branches were not present on all aspects of the tree due

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[1] Mention of trade names or products is for information only and does not imply endorsement by the U.S. Department of Agriculture.

to neighboring trees, but all aspects of the live canopy were systematically sampled. On each branch, the total length of the elongating terminal branch bud was measured to the nearest 1 mm in 1993 and 1994. For needle elongation, the length of 5 fascicles from petiole to tip were measured along one longitudinal axis of the whorl to the nearest 1 mm in the same years. Branchlet diameter was measured at the end of the growing season several mm above the growth scar between the previous and current year segments of the branchlet to the nearest mm. Chlorotic mottle and the number of annual whorls retained were measured monthly but reported here for early September on the same branches monitored for growth. This represents a maximum expression of injury for both characteristics. Total foliar chlorophyll content and a:b ratio, and *N* content were measured monthly during the growing season in 1994 and 1995 using the techniques described in Grulke et al. (1996). The seasonal maximum is reported here for mid July 1994. Total foliar *N* was measured (Carlo-Erba NA1500 C-N-S-analyzer) on a subsample of the tissue obtained for chlorophyll determination.

2.4. Root biomass determination

Root biomass was sampled from the same six trees measured for aboveground growth at each site. Conventional methods of root biomass determination were not effective in recovery of roots due to rocky soils (Vogt and Persson, 1991; Neill, 1992). Soil pits were excavated 1 m from ponderosa pine boles in mid July 1996 (SE quadrat) and in early September 1996 (SW quadrat). Root biomass was extracted as they were found, separated into size classes, cleaned, and placed in coolers for transport back to the laboratory. Roots were oven dried at 65°C for 48 h, and were dusted before weighing. The early July sampling was believed to represent a maximum, or near maximum root biomass based on soil pits examined monthly from May–October in 1994 and 1995. Because renewed allocation of carbohydrates to roots occurs in some individual trees at the end of September (Grulke, unpublished data), the below-ground root biomass was considered to be at a minimum in early September at all sites.

Litter was removed from the top of the mineral horizon (0–10 cm present). Pits were approximately 20×20 cm, and excavated successively by 20 cm depths to 60 cm, six per site. Pits were sometimes large due to removal of coarse rocks, and the true volume of the pit was determined incrementally (20 cm depths) by lining the pit with thin plastic and pouring in a known amount of water to the top of the mineral surface. In each 20 cm increment, roots were removed, identified, and separated into fine (<2 mm diameter fresh), medium (>2 mm, <1 cm), and coarse (>1 cm) size classes as found during extraction in the field, and bagged. Roots were later cleaned, dried, and weighed to the nearest 0.0001 g, but reported to the nearest 0.01 g due to high within site standard errors. Root biomass was expressed per soil volume (<2 mm fraction). The volume of pebbles, cobbles, and larger fragments was determined by the water displacement method.

The whole pit sampling method provided five fold (400 cm^2) the sampling volume of conventional coring taken at the same distance from the tree bole (78.5 cm^2, cores 10 cm in diameter) with greater recovery of all root size classes. From previous work, 90% of the root biomass was within the upper 80 cm of the top of the mineral horizon at these sites (C.R. Rose, unpublished data).

2.5. Statistical analyses

In all cases unless otherwise indicated, differences in tree response variables were tested with a 1-way analysis of variance. Values from individual trees were used as replication within a site. Significance is reported at the $p=0.05$ level.

Although many of the site attributes were taken in different years from when the root biomass was assessed, data were taken in years of average precipitation (1993, 1995, 1996) and a year of below average precipitation (1994). We assumed that if differences in site attributes were not different among sites between years with widely varying moisture status (a dominant environmental stressor in this mediterranean climate), their role in influencing root biomass differences across the pollution gradient was relatively minor.

3. Results and discussion

3.1. Patterns of root biomass across the pollution gradient

Fine and medium root biomass was significantly greater (a minimum of six fold) at the least polluted site (Camp Osceola, CO) relative to the more polluted sites (Strawberry Peak, SP, and Camp Paivika, CP) in early July (Table 4) in the upper 40 cm of mineral soil. Coarse root biomass was not correlated to biomass of fine (Adj. $r^2=0.021$) or medium (Adj. $r^2=0.085$) root biomass within the ped (20×20×20 cm). In early September, the biomass of both fine and medium roots was not significantly different across the gradient except for medium root biomass as a two point comparison between the intermediate (SP) and most polluted site (CP) at the 20–40 cm depth (Table 4).

Within a site, there were no significant differences between the upper (0–20 cm) and middle (20–40 cm) soil horizon for either the fine or medium root biomass in the July sampling (Table 5). At this sampling date, more

Table 4
Root biomass from 20×20 cm pits by depth (0–20, 20–40 and 40–60 cm) in early July and September 1996

Pollution level	Camp Osceola least	Strawberry Peak intermediate	Camp Paivika most	*F*	*p*
July					
0–20 cm					
fine roots	0.82 + 0.25a	0.13 + 0.02b	0.06 + 0.02b	8.244	0.004
medium roots	1.08 + 0.29a	0.02 + 0.01b	0.02 + 0.01b	13.41	0.001
coarse roots	0.63 + 0.42a	0a	0a	2.193	0.146
20–40 cm					
fine roots	0.81 + 0.25a	0.11 + 0.02b	0.06 + 0.01b	14.003	0.001
medium roots	1.81 + 0.34a	0.09 + 0.06b	0.03 + 0.01b	39.95	0.0001
coarse roots	0.61 + 0.50a	0.06 + 0.06a	0a	2.066	0.99
40–60 cm					
fine roots	0.27 (n = 1)a	0.11 + 0.03b	0.07 + 0.01b	5.028	0.031
medium roots	4.18 (n = 1)a	0.09 + 0.03b	0.02 + 0.01b	2206	0.0001
coarse roots	51.206 (n = 1)	0	0	–	–
September					
0–20 cm					
fine roots	0.13 + 0.02a	0.09 + 0.02a	0.08 + 0.05a	1.890	0.185
medium roots	0.14 + 0.02a	0.15 + 0.06a	0.04 + 0.01a	2.71	0.099
coarse roots	0	0	0	–	–
20–40 cm					
fine roots	0.08 + 0.05a	0.11 + 0.02a	0.11 + 0.02a	0.379	0.691
medium roots	0.13 + 0.06a	0.20 + 0.06a	0.10 + 0.02a	1.15	0.343
coarse roots	0	0	0	–	–
40–60 cm					
fine roots	0.03 (*n* = 1)a	0.10 + 0.01bc	0.09 + 0.01ac	3.372	0.076
medium roots	0.21 (*n* = 1)a	0.33 + 0.07ab	0.12 + 0.02ac	4.213	0.047
coarse roots	0 (*n* = 1)	0	0	–	–

Fine roots were < 2 mm in diameter, medium roots were > 2 mm, > 1 cm in diameter, and coarse roots were > 1 cm. Values given are mean ± 1 SE for the six trees measured per site unless sample size is otherwise indicated in parentheses. A one-way ANOVA was used to test for differences among sites, with the value of the *F* statistic and its significance (*p*) listed.
A *t*-test was used to test for differences between pairs of sites within a row.
Sites with differing letters were significant at the *p* = 0.05 level.

Table 5
Tests for differences in fine roots (FR) and medium roots MR) within a site between upper (0–20 cm) and middle (20–40 cm) soil horizons

		July		September	
		F	*p*	*F*	*p*
CO	FR	1.000	0.989	0.967	0.349
	MR	2.625	0.144	0.050	0.827
SP	FR	0.447	0.519	0.710	0.410
	MR	1.435	0.258	0.366	0.559
CP	FR	0.073	0.792	2.093	0.179
	MR	0.377	0.553	5.171	0.046

A one-way ANOVA was used to test for differences among sites, with six soil pits per site.
The *F* statistic and its signficance (*p*) are given. The three sites are listed in order of least to most polluted: Camp Osceola (CO), Strawberry Peak (SP), and Camp Paivika (CP).

medium than fine roots were present in the 0–40 cm profiles at the least polluted sites. In September, medium roots in the 0–20 cm soil horizon were significantly reduced relative to the 20–40 cm soil horizon, but only at the most polluted site.

Soil pits to 60 cm were sampled at Strawberry Peak and Camp Paivika, and the soil profiles had similar morphology (Table 6). Only one soil pit at Camp Osceola could be excavated to 60 cm. Coarse, fragmented bedrock was encountered at depths greater than 43–46 cm in the other pits at Camp Osceola. The proportion of medium to coarse rock fragments in the 0–40 cm profile was also greater at this site. One could argue that since the soil profile was rockier at the least polluted site (Camp Osceola), root biomass expressed on the basis of the fine soil fraction would be also be

Table 6
Soil profile morphology and chemical attributes

	Particle size	C/N	pH	P	K	Mg	Ca
Camp Osceola							
E, 0–8 cm	66/12/22	1.90/0.078	5.77	0.061	268	98	1075
B1, 8–20	66/8/26	0.97/0.043	5.85	0.030	233	98	796
B2, 20–59	69/8/23	0.58/0.031	5.99	0.021	237	89	621
Strawberry Peak							
A1, 0–26 cm	76/7/18	2.56/0.111	5.77	0.066	303	150	998
B1, 26–86	74/11/15	1.56/0.067	5.93	0.046	275	116	534
B2, 86–100	73/10/17	0.92/0.039	5.87	0.034	250	126	328
Camp Paivika							
A1, 0–8.5 cm	70/9/21	3.71/0.203	3.98	0.092	150	66	498
B1, 8.5–25	73/9/19	1.67/0.090	4.50	0.116	147	29	177
B21, 25–51	72/8/19	1.28/0.064	4.64	0.108	156	28	167
B22, 51–100	72/9/19	0.81/0.047	4.79	nd	180	37	220

Particle size is presented by sand/silt/clay, C/N is in % of each component, pH was measured in distilled water, P is in %, and the cations are presented in meq/dry wt.

greater. However, when fine or medium root biomass was expressed on the total pit volume excavated including the volume occupied by rocks in the upper and middle soil horizons, the differences in fine and medium root biomass between the three sites were still statistically significant for both fine and medium root, at both 0–20 and 20–40 cm depths ($p < 0.01$ for all comparisons cited here) in the early July sampling.

3.2. *Potential for environmental factors to affect root biomass patterns*

3.2.1. *Soil morphology and chemistry across the pollution gradient*

Aber et al. (1989) hypothesized that fine root biomass would be reduced in N-saturated forests in which the available soil N pool was predominantly nitrate. Our data from Camp Paivika clearly support this hypothesis. The forest at Camp Paivika is N-saturated based on a number of edaphic, plant, and hydrologic indicators (Fenn et al., 1996). Nitrate concentrations in soil extracts at Camp Paivika during the growing season ranged from 530 to 1800 μmol L^{-1} compared to 24 to 130 μmol L^{-1} at Camp Osceola. After 6 years of fertilization (50 and 150 kg ha^{-1} $year^{-1}$) in the Harvard Forest in Massachusetts, fine root biomass in a pine stand was lower in the high N treatment compared to the low N treatment, but not compared to the unfertilized control (Magill et al., 1997). In the hardwood plots, fine root biomass was increased by both levels of N fertilization relative to the control.

In the upper soil horizons at the most polluted site (Camp Paivika), nitrogen content was 2.6 fold greater than the least polluted site, and phosphorus was elevated as well. The entire soil profile at Camp Paivika is acidified relative to less polluted sites (Table 6). At the western end of the gradient, potassium, magnesium, and calcium were low relative to both the intermediate and eastern end of the gradient. The elevated potassium and magnesium levels in soil at Strawberry Peak may indicate increased cation deposition, and (or) insufficient anion (NO_3^-) and cation leaching to lower soil cation concentrations found at Camp Paivika.

Improved mineral nutrition generally improves growth of shoots more than roots (Cannell, 1989), and decreases the rate of turnover of fine roots (Norway spruce, *Picea abies* L. Karst; Nilsson and Wiklund, 1995; Majdi and Kangas, 1997), lowers root biomass (loblolly (*Pinus taeda* L.), and ponderosa pine: Griffin et al., 1995; red pine (*Pinus resinosa* Ait.): Haynes and Gower, 1995), and below-ground net primary production (Vogt et al., 1996), but has no effect on the timing of root growth (Tingey et al., 1996). Nitrogen fertilization lowers carbohydrate stores carried into the winter in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco; Margolis and Waring, 1986).

A biological response similar to that of elevated nitrogen supply to the soil was found with exposure to elevated ozone: in the spring following exposure to elevated ozone, lower root biomass was found in ponderosa pine seedlings (Andersen et al., 1991). In our study, there were significant differences in N deposition between the most polluted site (CP) and the intermediate pollution site (SP) (Fenn et al., 1996), with no significant difference in fine or medium root biomass in the early July sampling date. From this, we can conclude only that either the N deposition level at SP is sufficiently high (above a threshold) to lower fine and coarse root biomass, or that ozone exposure dominated the stress response.

In the summer there is continual N deposition to the surface litter. In early September, medium roots were significantly lower in the 0–20 versus the 20–40 cm depth at the most polluted site. Fine root biomass was similar at both intermediate and the most polluted sites. We could conclude that N accumulation in the upper soil horizon limits root growth at only the most polluted site as it accumulates through the Mediterranean summer, but there is no evidence to support transfer of nitrate or ammonium to the mineral horizon from the litter with out an autumn precipitation event (M. Poth, unpublished data).

3.2.2. Soil moisture across the pollution gradient

The snow pack provides 90% of the available moisture annually at these sites (Miller, 1992). Summer precipitation is rare, and if it does occur, generally does not moisten the mineral soil appreciably (Temple and Miller, 1998). In their study, if precipitation occurred after the onset of mid-summer drought, ponderosa pine did not appear to respond, based on needle xylem pressure potentials and stomatal conductance.

Drought stress may truncate root growth, but in both 1995 (year prior to root sampling) and 1996 (year of root sampling), there was above average precipitation (based on a 30 year average from the Big Bear Dam in the eastern San Bernardino Mountains, San Bernardino County, Flood Control District, annual data sets). Even in a drought year (1994), needle elongation growth was not truncated until the end of July, which was not expected to have affected the root biomass sampled in early July of a mesic year.

Late summer drought and low soil moisture content decreased ponderosa pine fine root biomass within a growing season in central Oregon (Hermann and Petersen, 1969). In our study, trees at the least polluted site (CO) developed lower pre-dawn xylem potential by early September consistently for three years (1993–1995; Fig. 1), but at this early fall sampling date, fine and medium root biomass was not significantly different across the sites. Root biomass was significantly different in early July 1996 at a time when drought stress had not yet developed at any of the sites. The influence of long-term soil moisture differences among the sites on tree allocation patterns may have contributed to the patterns of root growth observed. However, fine root turnover is especially dynamic (Tingey et al., 1996), and would be expected to respond to short-term (within the growing season) differences in site water status.

If the lower soil horizon is rockier than the upper soil horizons (as at Camp Osceola), one could argue that the root biomass would be concentrated in the upper soil horizons relative to less rocky sites (i.e. Strawberry Peak and Camp Paivika). Although the upper soil horizons dry out by mid-July even in a mesic year (upper 40 cm < 1.5% gravimetric soil moisture, Grulke, unpubl. data; Temple and Miller, 1998), trees at all of the sites access deeper water sources after mid-July. There were no significant differences in pre-dawn xylem potential among sites by early September in the driest year, 1994 ($F=3.64$; $p=0.064$; Fig. 1). Apparently, more soil volume is available to roots through fragmented

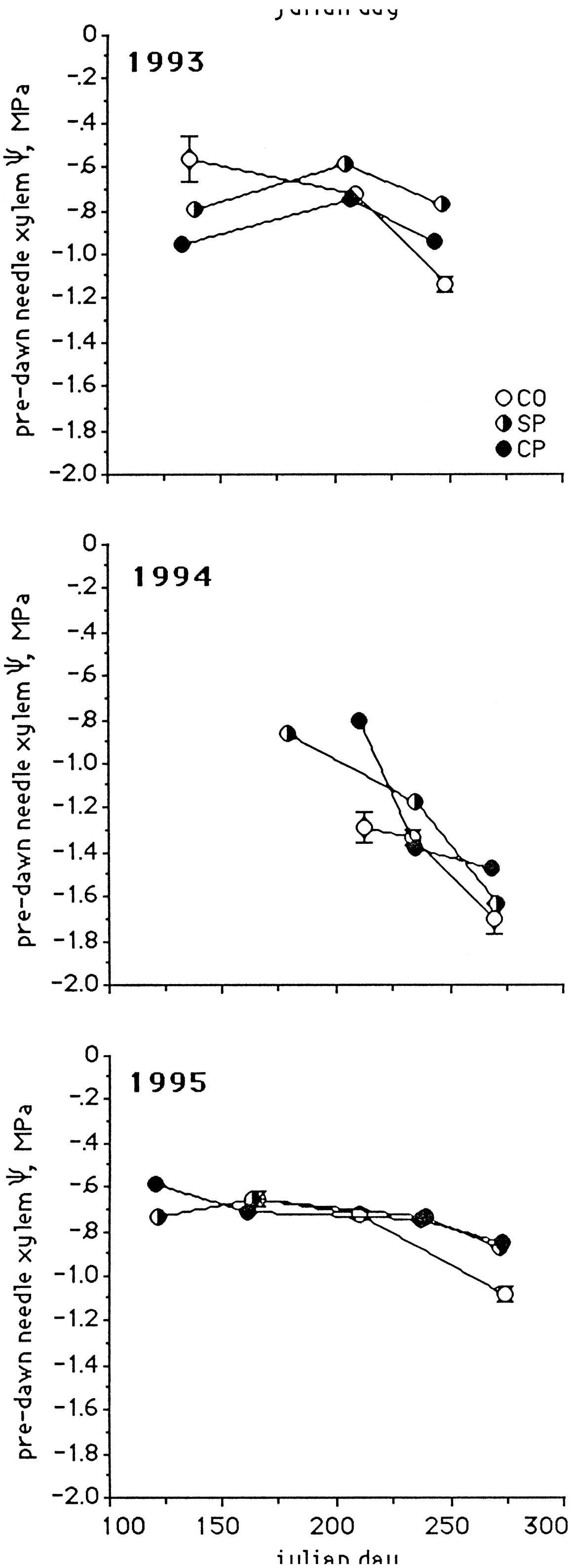

Fig. 1. Seasonal trend of pre-dawn needle xylem water potential in MPa measured monthly during the growing season during the 3 years prior to root mass measures. This measure of water status was used as a integrative measure of soil water available to the tree. Unless otherwise indicated, ±1 SE falls within the symbol indicating the mean of the six trees at each site.

bedrock than can be conventionally determined (Arkley, 1981; Jones and Graham, 1993).

3.2.3. *Soil temperature across the pollution gradient*

Low soil temperatures may limit root biomass in woody plants (Kozlowski and Pallardy, 1997). Soil temperatures at the high pollution site (CP) were favorable for root growth by early March, while soil temperatures lagged behind by approximately 1–2 weeks at the less polluted sites (SP, CO; Julian day 105; Fig. 2). There was little difference in soil temperatures between the intermediate (Strawberry Peak) and eastern (Camp Osceola) sites in the first half of the year (Julian day 1–180). Root biomass was approximately six fold greater at Camp Osceola relative to Strawberry Peak and Camp Paivika. Although a shorter growing season might result in a stronger pulse of root growth at the CO site, at the time of sampling, root biomass would be expected to be the greatest at the most polluted site (CP) rather than the least polluted site (CO). Therefore, we discounted soil temperature as a major contributing factor to the observed response in root biomass.

3.3. *Potential for biotic factors to affect root biomass patterns*

3.3.1. *Above-ground phenology across the pollution gradient*

Differences in root biomass between sites could be attributed to potential differences in phenology across the San Bernardino Mountains. Although root growth often continues during shoot elongation, above-ground phenological events can be used to bracket periods of maximum root growth. For ponderosa pine in central Oregon, root growth is first initiated after snow melt which then allows bud break, then branch bud growth, and finally needle elongation (Hermann and Petersen, 1969). Carbon allocation to branch and needle growth is thought to limit allocation to fine root production in slash pine (*Pinus elliotii* Engelm., Gholz and Cropper, 1991), and generally in woody plants (Kozlowski and Pallardy, 1997). Across the San Bernardino Mountains, snow melt generally occurs from February to mid May (Miller, 1992). However, soil temperatures at −10 cm are greater than 5°C for all but two months at the most polluted site, and at all sites by April (Fig. 2). Here we use aboveground phenology of branchlet and needle elongation growth as a proxy for the potential for variability in timing of below-ground growth.

In 1993, bud break occurred in early May at approximately the same time across all sites within the pollution gradient (Fig. 3). In 1994, bud break occurred by late May at the more westerly CP and SP sites, and approximately one week later at CO. Branch elongation growth was 90% complete by the last week of June at all sites, in all three years (1993, 1995: mesic, cool years; 1994: xeric, warmer year). Needle elongation growth was more variable between years, reaching 90% of maximum growth by the second week of August in the mesic years but was truncated in the xeric year by the last week of July at all three sites. Because the phenology shifted one (bud break) to three weeks (cessation of needle elongation) between years, the timing of branch and needle growth appears to respond to regional environmental conditions between years, not microenvironmental variations between sites. The one week difference in the timing of bud break may influence root biomass, however it was discounted as a major factor contributing to the six fold difference as found in this study.

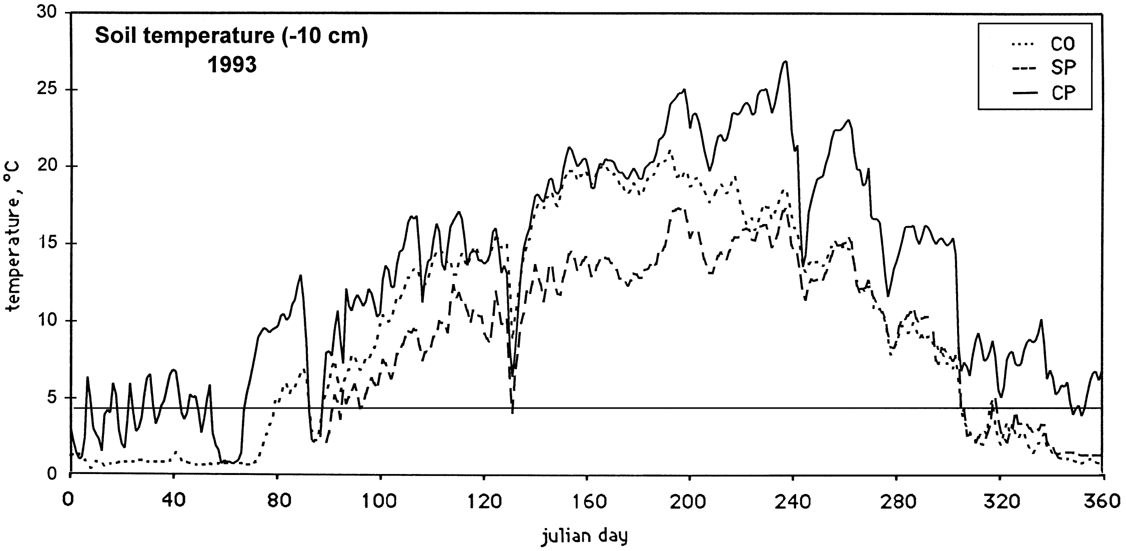

Fig. 2. Seasonal trend of soil temperature taken at −10 cm for 1993 across the three sites in the San Bernardino Mountains.

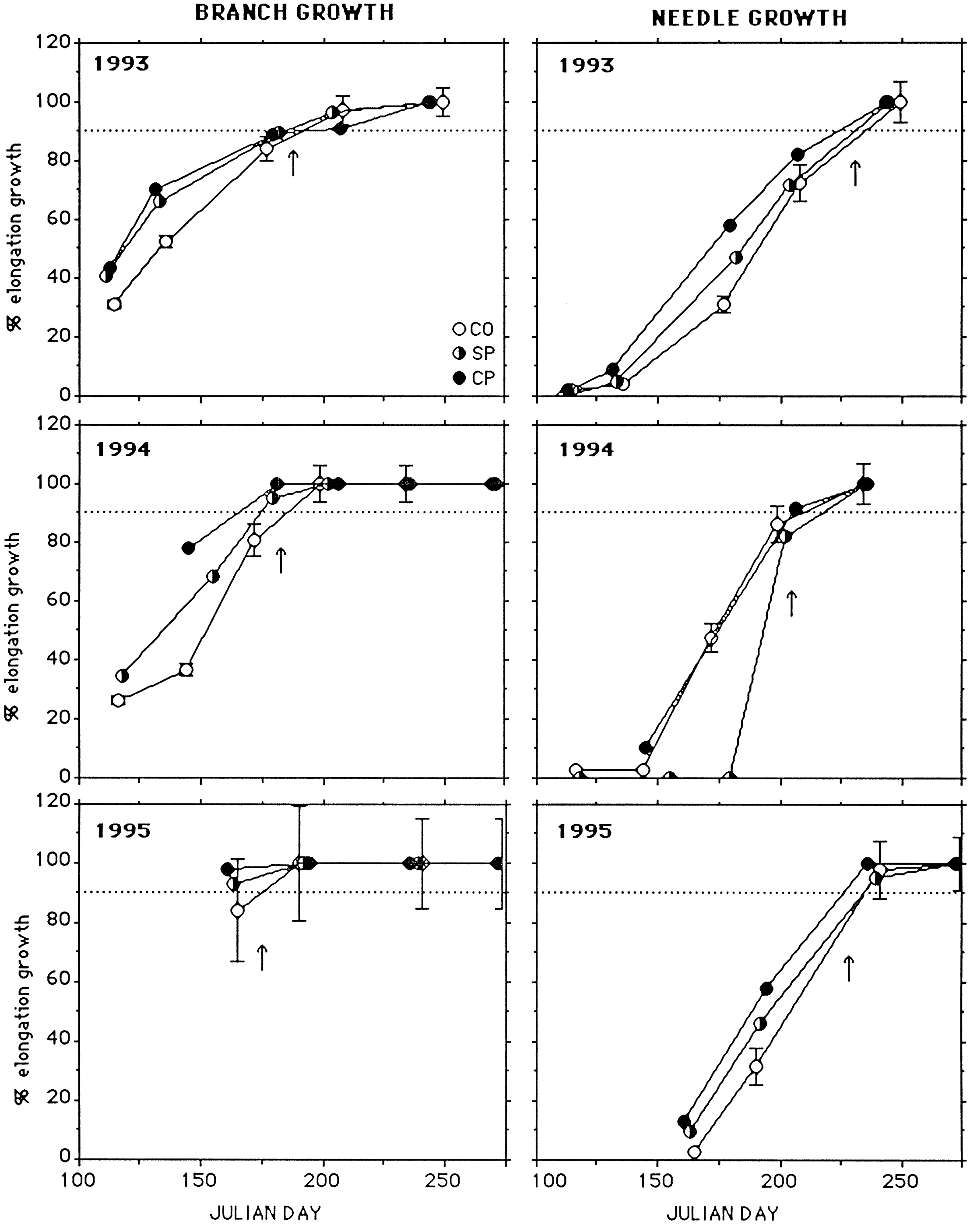

Fig. 3. Synchronization of branch and needle elongation across the pollution transect. Dotted line and arrows indicate 90% of total elongation growth seasonally. Each point represents the mean of six trees at each site. Camp Osceola (CO), Strawberry Peak (SP), and Camp Paivika (CP) represent a gradient from least to most polluted.

3.3.2. Tree density and canopy shading among sites across the pollution gradient

The distance to the nearest neighboring tree is a measure of the inferred level of competition an individual tree experiences. At the least polluted site (CO), there were clumps of trees separated by open spaces. Although tree density varied among the three sites, the distance to the nearest conspecific tree was not signficantly different among the three sites (Table 3).

Shading lowers carbon acquisition, and results in greater allocation of carbohydrates to shoots over roots. Chlorophyll a:b ratios of the canopy foliage did not suggest differences in the light environment the study trees were exposed to across the pollution gradient.

Foliar injury (percentage chlorotic mottle) and the number of annual whorls retained were significantly different across the pollution gradient (Table 3). Total foliar chlorophyll content was not significantly different across the pollution gradient in 1994 (a drought year), but was greater at the most polluted site in 1995 (above average precipitation). The high N deposition, combined with moisture may permit N uptake at a rate sufficient to maintain reparation activities at this site.

Although tree density was greatest at Strawberry Peak, the intermediate site, proximity to the nearest tree was highest at Camp Osceola, the least polluted site. If shading were a significant factor among sites, root biomass would have been lower, not higher at Camp Osceola. Tingey et al. (1996) demonstrated reduced leaf area index and fine root density with increased shading of ponderosa pine grown in open-top chambers. The proportion of foliage to aboveground woody tissue was greatest at 0.19 ± 0.05 at the least polluted site (CO) relative to 0.06 ± 0.02 for SP, and 0.10 ± 0.03 at CP based on a subsample of three average trees in the 20–40 year age class at each site in 1993 (Grulke, unpubl. data set).

4. Conclusions

We analyzed above- and below-ground responses of ponderosa pine along a known gradient ranging from high ozone exposure and N deposition to moderate ozone exposure and low N deposition across the San Bernardino Mountains. We demonstrated that high foliar injury and lower foliar retention accompanied lower root biomass as a function of the gradient in long-term pollution deposition. We conclude that long-term exposure to oxidant pollution and nitrogen deposition appear to be the dominant factors influencing the pattern of root biomass across the pollution gradient examined in this study. This conclusion is based on analysis of other site factors known to affect root growth across the gradient, and their possible contribution to the patterns observed. The results suggest that site differences in edaphic and biological characteristics cannot explain the observed responses in patterns of root growth. In addition, the biological and environmental factors did not co-vary in paired comparisons between the three sites. The most likely explanation for the lower fine and medium root biomass observed in forest stands across this gradient is long-term anthropogenic ozone exposure and N deposition. Lower root biomass in high pollution areas may pose a long-term threat to forest sustainability if the capacity of ponderosa pine trees to withstand multiple stresses is compromised.

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